


Fission yeast strains and general techniques

TF Tomoyuki Fukuda KS Kazuhiro Shiozaki

Updated date: Oct 27, 2020

 An abbreviated version of this protocol was published in eLIFE in Dec 2017

Ragulator and GATOR1 complexes promote fission yeast growth by attenuating TOR complex 1 through Rag GTPases

DOI: 10.7554/eLife.30880

Detailed protocol

Cells were grown in 180 ml of EMM liquid medium in a 500-ml flask at 30°C with continuous shaking at 170 rpm until the cell density reached $OD_{600} = 0.2$. A 45-ml aliquot of the culture was taken for the “0 min” sample and fixed by mixing with 5 ml solution of trichloroacetic acid (TCA; final concentration = 10%). The remaining culture was then filtered onto a mixed cellulose ester membrane (ϕ 47 mm, pore size 0.45 μ m; Advantec, USA), and the harvested cells on the membrane were rinsed with 100 ml of EMM-N (EMM without NH_4Cl) to initiate nitrogen starvation. The washed cells were immediately resuspended in pre-warmed 135 ml of EMM-N liquid medium in a 500-ml flask and incubated at 30°C with continuous shaking at 170 rpm. At each time point after nitrogen starvation, a 45-ml aliquot of the culture was harvested and fixed with 5-ml TCA.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fukuda, T. and Shiozaki, K. (2020). Fission yeast strains and general techniques. Bio-protocol Preprint. bio-protocol.org/prep576.
2. Chia, K. H., Fukuda, T., Sofyantero, F., Matsuda, T., Amai, T. and Shiozaki, K. (2017). Ragulator and GATOR1 complexes promote fission yeast growth by attenuating TOR complex 1 through Rag GTPases. eLIFE. DOI: [10.7554/eLife.30880](https://doi.org/10.7554/eLife.30880)

Copyright: Content may be subjected to copyright.